



TARPTAUTINIŲ MOKSLO IR TECHNOLOGIJŲ
PLĖTROS PROGRAMŲ AGENTŪRA



A possible threat to the timber industry: '*Candidatus Phytoplasma pini*' in Scots pine (*Pinus sylvestris* L.) in Lithuania



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'*Candidatus Phytoplasma pini*' spread in Europe

Germany

Schneider *et al.*, *Int. J. Syst. Evol. Microbiol.*, 55, 303–307. 2005

Spain

Poland

Śliwa *et al.*, *J. Phytopath.*, 156, 88–92. 2008

Czech Republic

Genbank database

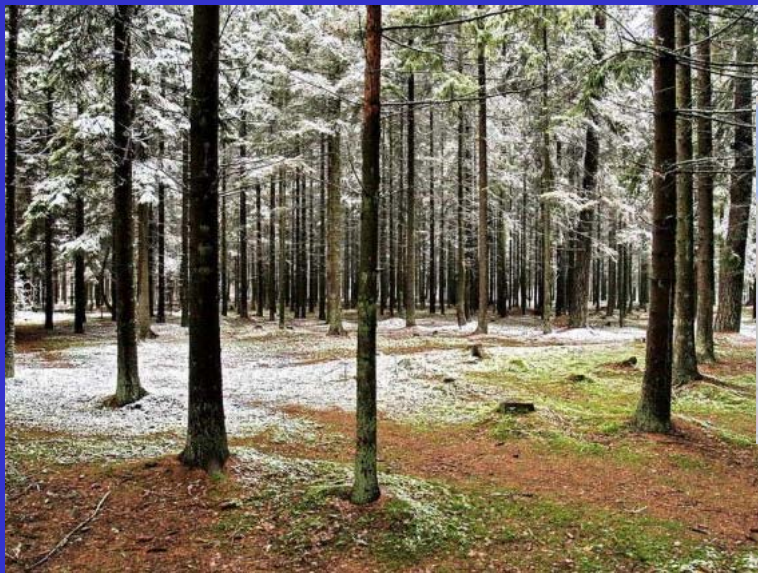


A possible threat to the timber industry

The timber industry generates about 2% of industrial production and engages 13% of the workforce in Lithuania.

Timber industry: furniture, paper, firewood, cork, wood-pulp.

In 2008, in Southern Lithuania, we noticed several diseased pine trees with unusual symptoms similar to those caused by phytoplasmas.



Range of *Pinus sylvestris*

**The observed pine trees (*Pinus sylvestris* L.)
in Lithuania exhibited**

excessive branching, dwarfed needles and dry shoots.



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Material and Methods of identification

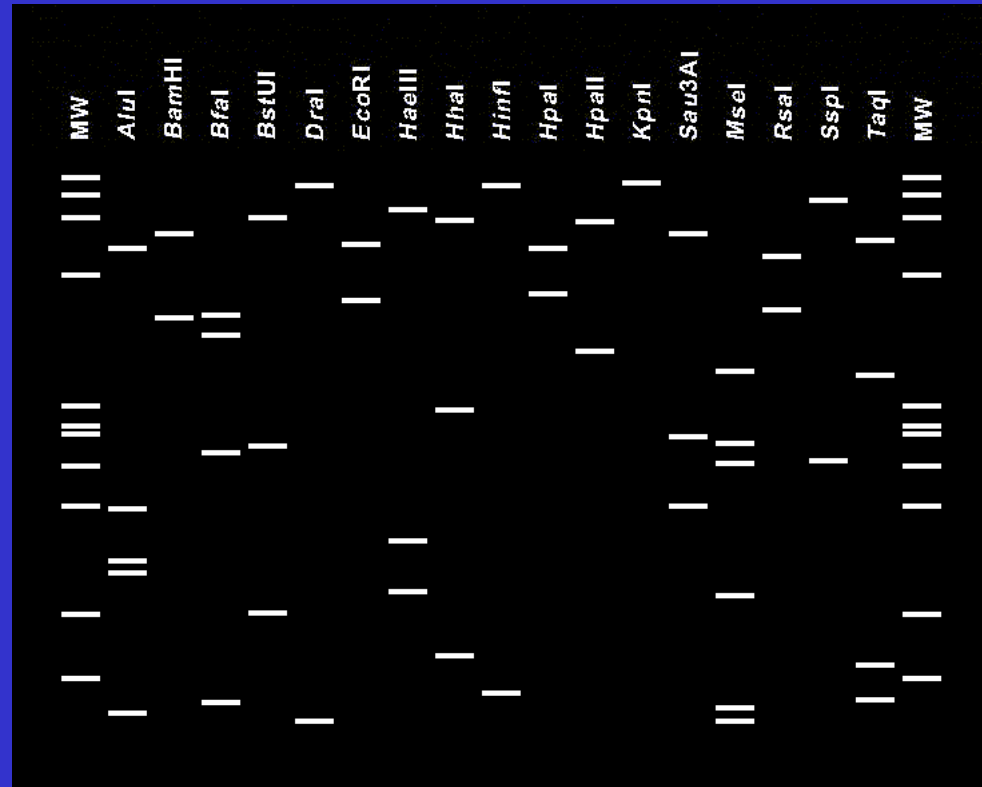
DNA from dwarfed needles was extracted using **Genomic DNA Purification Kit (Fermentas, Lithuania)** according to manufacturer's instructions.

Nested PCR assays using extracted DNA, primer pairs **P1/R16-SR** (Deng and Hiruki., 1991; Lee *et al.*, 2004) and **R16F2n/R16R2n** (Lee *et al.*, 1998), and **AmpliTaq Gold polymerase** (Applied Biosystems, USA) were carried out, and the amplified products were analysed as previously described (Lee *et al.*, 1998).

Products from nested PCR primed by R16F2n/R16R2n were analysed by single enzyme digestion (**RFLP analysis**).

The PCR product primed by R16F2n/R16R2n was cloned in *E. coli* using the TOPO-TA cloning kit (Invitrogen, USA) and **sequenced**.

'*Ca. Phytoplasma pini*' (16SrXXI-A) in Lithuania



Identification / classification based on RFLP analysis of 1.2 kb of 16S rDNA; virtual gel image.

16S rDNA Sequence Analysis

Percent sequence identity with '*Ca. Phytoplasma pini*':

<i>'Ca. P. pini'</i> EF128037 Poland	AJ310849 Germany	AJ632155 Spain
100%	99.9%	99.9%

Sequence identity of Pine bunchy top (PineBT) acc. no. GU289676
phytoplasma is

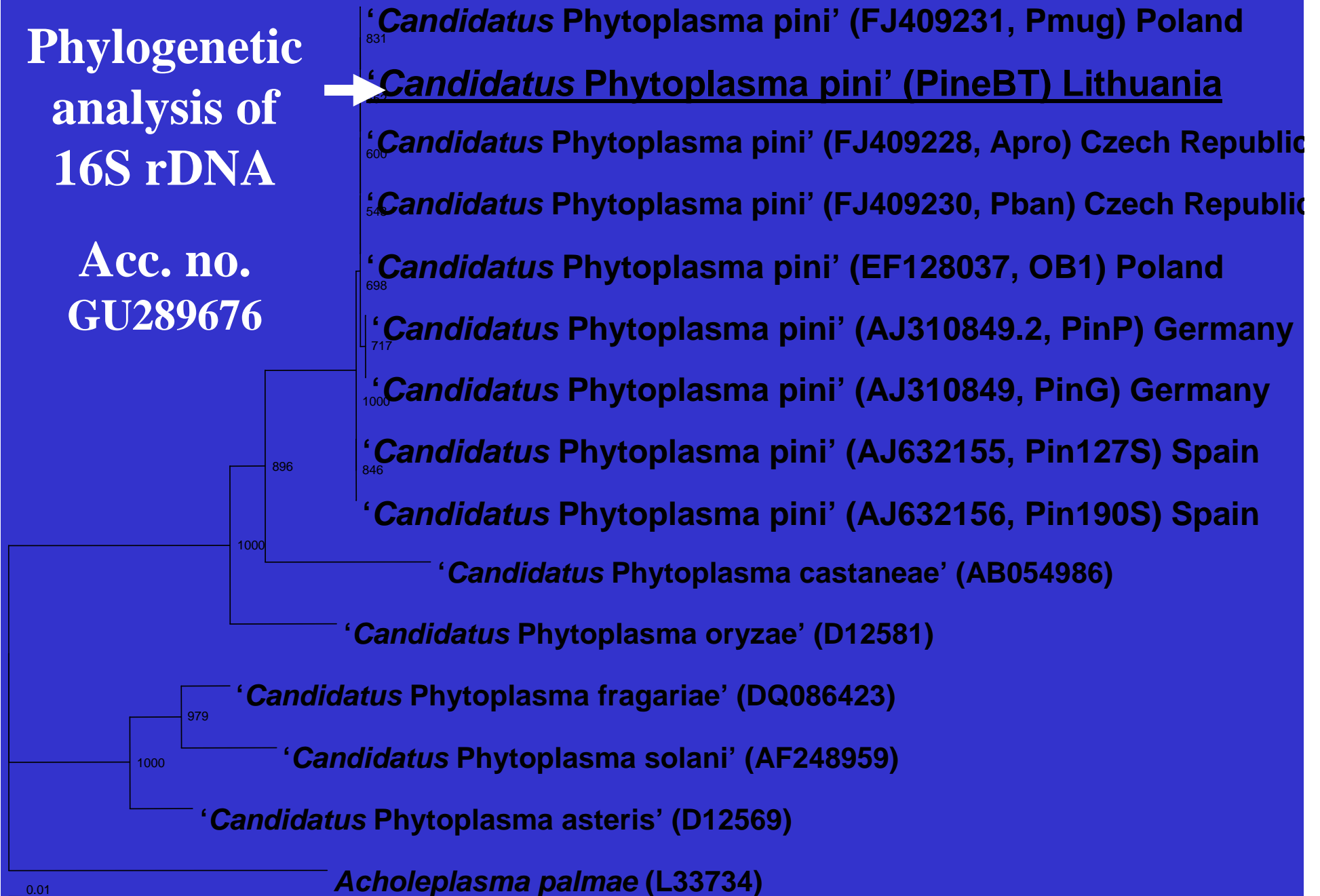
100-99.9 %

with other '*Ca. P. pini*' strains.

Therefore, PineBT phytoplasma is strain of '*Ca. P. pini*'

Phylogenetic analysis of 16S rDNA

Acc. no.
GU289676



Concluding Remarks

- 1. Sequence and RFLP analysis of 16S rDNA indicated that the tested symptomatic Scots pine trees are infected by '*Ca. Phytoplasma pini*' in Lithuania.**
- 2. The phytoplasmal disease of pine trees possibly can cause tangible losses in the wood industry.**
- 3. This is the first report of '*Ca. Phytoplasma pini*' in Lithuania.**



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'*Candidatus Phytoplasma pini*' in Scots pine
(*Pinus sylvestris* L.) in Lithuania**

Thank you for your attention !

